## APPENDIX B: AUTOCLAVE QUALITY CONTROL

## QUALITY ASSURANCE PROGRAM DNA TYPING OF BIOLOGICAL MATERIALS - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION VI

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Due to the sensitive nature of DNA analysis, especially PCR applications, it is necessary to ensure adequate sterilization of glassware and solutions. Evaluation of the efficiency of sterilization is best accomplished by the utilization of a biological indicator. The spores of the thermophile *Bacillus Stearothermophilus ATCC 7953* grow during incubation at 56-60°C, but are inactivated if exposed to temperatures above 125°C. Acid production during the growth of the bacteria will cause a colormetric change in the indicator solution from purple to yellow. It is necessary to use a positive control to show growth of the bacteria during normal incubation without inactivation by sterilization. The Barnstead/Thermolyne Ampules are to be stored at 15° to 30°C prior to use.

<u>Precaution: The ampules contain live cultures. Handle with care to prevent breakage. Do not store in refrigerator with other biologicals. Ampules may not be used after expiration date. Use each ampule once and destroy by autoclaving or incineration after testing.</u>

## **Biological Indicators for Steam Sterilization**

Purchased from FISHER, Catalog number 14-490-25.

## Procedure:

- 1. Label ampule(s) "Test" and "Control".
- 2. Carefully place one unopened purple ampule (Test) into the center of the autoclave.
- 3. Proceed with sterilization as indicated by the manufacturer for a liquid solution or empty glassware.
- 4. After the cycle has ended, allow the ampule to cool for 10 to 30 minutes until it is cool enough to handle. To activate the media, place the ampule in an upright position in a plastic crusher. Gently squeeze the crusher to break the glass ampule. Break both the "Test" and "Control" ampules. Place "Test" and "Control" ampules into a 56-60°C incubator or oven.
- 5. Observe ampules at 24 and 48 hours for color change from purple to yellow.
  - The "Control" ampule should change after approximately 24 hours of incubation. If the "Test" ampule changes within the 48 hour maximum, the sterilization cycle fails to meet the proper standard
  - The sterilization cycle passes the test if the "Test" ampule does not turn yellow within the 48 hour incubation period but the "Control" ampule changes color.
- 6. Repeat test with new ampules if the "Control" does not change color from purple to yellow.
- 7. Record the results of the test. If the test fails, remove the autoclave from service and contact an authorized vendor to service the autoclave.

**♦END**